FILE 'MEDLINE, CAPLUS, BIOSIS, EMBASE' ENTERED AT 13:38:08 ON 10 MAR 2005

L1	232953	S (ANTIGEN PRESENTING CELL?) OR (APC) OR (DENDRITIC CELL) OR (D
L2	11587	S L1 (S) ((LOAD?) OR (PULSE?))
L3	19130	S ELECTROPOR?
L4	2875	S ELECTROFUS?
L5	3	S ELECTROPERMEABLIZ?
L6	1376	S ELECTROPERMEABILIZ?
L7	80	S L2 (S) L3
L8	48	DUP REM L7 (32 DUPLICATES REMOVED)
L9	34	S L8 (P) (ANTIGEN OR PROTEIN)
L10	51	S L2 (S) L4
L11	35	DUP REM L10 (16 DUPLICATES REMOVED)
L12	5	S L2 (S) L6
L13	5	DUP REM L12 (0 DUPLICATES REMOVED)
L14	8	S ELECTROPORAT? FLOW
L15	8	DUP REM L14 (0 DUPLICATES REMOVED)
L16	721	S L2 (S) LYSATE
L17	268	S L16 (S) ANTIGEN
L18	205	S L17 (S) (TUMOR OR TUMOUR)
L19	2	S L18 (S) ELECTRO?

AU Kim, Kwang-Woon [Reprint author]; Kim, Sun-Hee [Reprint author]; Jang, Jung-Hee [Reprint author]; Lee, Eun-Yup; Park, Soon-Won [Reprint author]; Um, Jee-Hyun; Lee, Yun-Jung [Reprint author]; Yee, Sung-Tae; Seo, Su-Yeong; Yoon, Sik; Lee, Chang-Hun; Park, Yeung-Min; Chung, Byung-Seon [Reprint author]; Kang, Chi-Dug

SO FASEB Journal, (March 20, 2002) Vol. 16, No. 4, pp. A664. print.
Meeting Info.: Annual Meeting of the Professional Research Scientists on Experimental Biology. New Orleans, Louisiana, USA. April 20-24, 2002.
CODEN: FAJOEC. ISSN: 0892-6638.

QH301.F4

TI Enhancement of antitumor immunity by dendritic cells loaded with exogenous antigen by electroporation.

AB To develop an efficient antitumor immunotherapy, we have examined if dendritic cells (DCs) loaded with antigens by electroporation (EP) present more antigens via MHC class I pathway. Ovalbumin (OVA) was effectively loaded into DCs by EP. DCs loaded with OVA by EP presented more MHC class I-restricted determinants compared with DCs incubated with OVA. When electroporated DCs were incubated with OVA for additional times, both MHC Class I- and II-restricted presentation of OVA were increased compared with each single procedure including EP or simple incubation with OVA. Immunization with DCs loaded with OVA by EP induced higher cytotoxicity of splenocytes to E.G7 cells, a clone of EL4 cells transfected with an OVA cDNA, than immunization with DCs incubated with OVA. In the animal study, immunization with DCs loaded with OVA by EP induced an effective preventive antitumor immunity against tumor of E.G7 cells. In addition, immunization with DCs loaded with antigen by combination of EP and incubation, which enhance stimulation of both CD4+ and CD8+ T cells, protected completely mice from tumor formation, and prolonged survival. These results demonstrated that EP would be a useful way to enhance the MHC class I-mediated antitumor immunity, and the combination of EP and incubation could be a simple and efficient method to introduce soluble antigens into both MHC Class I- and II- pathway and consequently lead to induction of strong antitumor immunity.

AU Kim Kwang-Woon; Kim Sun-Hee; Jang Jung-Hee; Lee Eun-Yup; Park Soon-Won; Um Jee-Hyun; Lee Yun-Jung; Lee Chang-Hun; Yoon Sik; Seo Su-Yeong; Jeong Min-Ho; Lee Seong-Tae; Chung Byung-Seon; Kang Chi-Dug

SO Cancer immunology, immunotherapy : CII, (2004 Apr) 53 (4) 315-22.
Electronic Publication: 2003-12-18.

Journal code: 8605732. ISSN: 0340-7004.

TI Dendritic cells loaded with exogenous antigen by electroporation can enhance MHC class I-mediated antitumor immunity.

AB To develop an efficient antitumor immunotherapy, we have examined if dendritic cells (DCs) loaded with soluble antigens by electroporation present more antigens via the MHC (major histocompatibility complex) class I pathway, which mediate a cytotoxic T-cell response. DCs loaded with ovalbumin (OVA) by electroporation presented more MHC class I-restricted determinants compared with DCs pulsed with OVA. When electroporated DCs were pulsed with OVA for additional times, both MHC class I- and II-restricted presentation of OVA were increased compared with each single procedure, including electroporation or simple pulse. Immunization with DCs loaded with OVA by electroporation induced higher cytotoxicity of splenocytes to E.G7 cells, a clone of EL4 cells transfected with an OVA cDNA, than immunization with DCs pulsed with OVA. In the animal study, immunization with DCs loaded with OVA or tumor cell lysates by electroporation induced an effective antitumor immunity against tumor of E.G7 cells or Lewis lung carcinoma cells, respectively. In addition, immunization with DCs loaded with antigen by combination of electroporation and pulse , completely protected mice from tumor formation, and prolonged survival, in both tumor models. These results demonstrated that electroporation would be a useful way to enhance MHC class I-mediated antitumor immunity without functional deterioration, and that the combination of electroporation and pulse could be a simple and efficient antigen-loading method and consequently lead to induction of strong antitumor immunity.

IN Liu, Linda N.; Weiss, Jonathan M.

SO PCT Int. Appl., 62 pp.
CODEN: PIXXD2

TI Loading of APC with antigens by electroporation

AB The authors disclose loading an antigen-presenting cell (APC) with one or more antigens by electroporation. In one example, an augmented anti-tumor response was shown in mice receiving dendritic cells electroporated with a lysate from renal cell carcinoma cells.

AU Chen W; Carbone F R; McCluskey J

SO Journal of immunological methods, (1993 Mar 15) 160 (1) 49-57.
Journal code: 1305440. ISSN: 0022-1759.

TI Electroporation and commercial liposomes efficiently deliver soluble protein into the MHC class I presentation pathway. Priming in vitro and in vivo for class I-restricted recognition of soluble antigen.

AB Class I major histocompatibility complex (MHC)-restricted cytotoxic T lymphocyte responses to ovalbumin (OVA) were evaluated following delivery of soluble antigen mixed with commercial liposomes or by electroporation of soluble protein into target cells. Splenic antigen presenting cells (APC) and transfected L cell lines were sensitised for recognition by OVA-specific, class I-restricted T hybridomas when antigen was introduced by either method into live cells. Delivery of soluble OVA by both electroporation and commercial liposomes proved more efficient than osmotic loading in sensitising for class I presentation. OVA-specific cytotoxic T lymphocytes (CTL) were effectively primed in naive mice following reinjection of spleen cells pulsed with soluble OVA encapsulated by liposomes or electroporated in vitro. These CTL recognised the well defined OVA257-264 determinant in association with H-2Kb and were derived under conditions where CTL activity obtained from cross priming by soluble

OVA alone was undetectable. In addition, using electroporation and commercial liposomes, the loading of APC for OVA recognition required intact MHC-linked antigen presentation genes deleted in the T2-Kb cell line. Antigen delivery to APC by electroporation and commercial liposomes provides a simple and efficient way of studying class I-restricted T cell recognition of soluble protein antigens.

AU Scott-Taylor T.H.; Pettengell R.; Clarke I.; Stuhler G.; La Barthe M.C.; Walden P.; Dalgleish A.G.

SO Biochimica et Biophysica Acta - Molecular Basis of Disease, (2000) 1500/3 (265-279).
Refs: 60
ISSN: 0925-4439 CODEN: BBADEX

TI Human tumour and dendritic cell hybrids generated by electrofusion: Potential for cancer vaccines.

AB Hybrid cells created by fusion of antigen presenting and tumour cells have been shown to induce potent protective and curative anti-tumour immunity in rodent cancer models. The application of hybrid cell vaccines for human tumour therapy and the timely intervention in disease control are limited by the requirement to derive sufficient autologous cells to preserve homologous tumour antigen presentation. In this study, the efficiency of various methods of electrofusion in generating hybrid human cells have been investigated with a variety of human haemopoietic, breast and prostate cell lines. Cell fusion using an electrical pulse is enhanced by a variety of stimuli to align cells electrically or bring cells into contact. Centrifugation of cells after an exponential pulse from a Gene Pulser electroporation apparatus provided the highest yield of mixed cell hybrids by FACS analysis. An extensive fusogenic condition generated in human cells after an electrical pulse contradicts the presumption that prior cell contact is necessary for cell fusion. Alignment of cells in a concurrent direct current charge and osmotic expansion of cells in polyethylene glycol also generated high levels of cell fusion. Waxing of one electrode of the electroporation cuvette served to polarise the fusion chamber and increase cell fusion 5-fold. Optimisation of a direct current charge in combination with a fusogenic pulse in which fusion of a range of human cells approached or exceeded 30% of the total pulsed cells. The yield of hybrid prostate and breast cancer cells with dendritic cells was similar to the homologous cell fusion efficiencies indicating that dendritic cells were highly amenable to fusion with human tumour cells under similar electrical parameters. Elimination of unfused cells by density gradient and culture is possible to further increase the quantity of hybrid cells. The generation and purification of quantities of hybrid cells sufficient for human vaccination raises the possibility of rapid, autologous tumour antigen presenting vaccines for trial with common human tumours.

AU Goddard R V; Prentice A G; Copplestone J A; Kaminski E R

SO Clinical and experimental immunology, (2003 Jan) 131 (1) 82-9.
Journal code: 0057202. ISSN: 0009-9104.

TI In vitro dendritic cell-induced T cell responses to B cell chronic lymphocytic leukaemia enhanced by IL-15 and dendritic cell-B-CLL electrofusion hybrids.

AB HLA class II-restricted proliferative and cytotoxic T cell (CTL) responses to B cell chronic lymphocytic leukaemia (B-CLL) can be generated using autologous dendritic cells (DCs) pulsed with tumour cell lysate. In this study a number of different approaches were used to optimize further the in vitro system. First, the effects of a variety of maturation agents were studied. The addition of TNF-alpha, polyriboinosinic polyribocytidylic acid (Poly(I:C)) and LPS to autologous DCs resulted in

the emergence of only a small percentage of CD83+ DCs, IFN-alpha having no demonstrable effect. Only the addition of Poly(I:C) to DCs resulted in modestly increased specific cytotoxicity to B-CLL targets, IFN-alpha and LPS having no effect. Secondly, T cells were pretreated with IL-15, prior to culturing with lysate-pulsed autologous DCs. A significant increase in T cell activation (P = 0.038), IFN-gamma secretion (P = 0.030) and specific cytotoxicity to B-CLL targets (P = 0.006) was demonstrated compared to untreated T cells. Thirdly, monocyte derived DCs electrofused with B-CLL B cells were compared with lysate-pulsed DCs. T cells stimulated by fused DCs generated higher levels of specific cytotoxicity to autologous B-CLL B cell targets than those stimulated by lysate pulsed DCs (P = 0.013). Blocking studies demonstrated inhibition of this cytotoxicity by both anti-CD4 (P = 0.062) and anti-CD8 monoclonal antibodies (P = 0.018), suggesting the generation of both HLA class I- and HLA class II-restricted CTL responses. In summary, in vitro B-CLL-specific T cell responses can be enhanced further by preincubating T cells with IL-15 and using autologous fused DC-B-CLL hybrids instead of autologous lysate-pulsed DCs. These preliminary data require confirmation with larger numbers of patients. Such an approach, however, may eventually provide effective immunotherapy for treatment of B-CLL.

AU Parkhurst M.R.; DePan C.; Riley J.P.; Rosenberg S.A.; Shu S.
SO Journal of Immunology, (15 May 2003) 170/10 (5317-5325).
Refs: 64
ISSN: 0022-1767 CODEN: JOIMA3
TI Hybrids of dendritic cells and tumor cells generated by electrofusion simultaneously present immunodominant epitopes from multiple human tumor-associated antigens in the context of MHC class I and class II molecules.
AB Hybrid cells generated by fusing dendritic cells with tumor cells (DC-TC) are currently being evaluated as cancer vaccines in preclinical models and human immunization trials. In this study, we evaluated the production of human DC-TC hybrids using an electrofusion protocol previously defined for murine cells. Human DCs were electrically fused with allogeneic melanoma cells (888mel) and were subsequently analyzed for coexpression of unique DC and TC markers using FACS and fluorescence microscopy. Dually fluorescent cells were clearly observed using both techniques after staining with Abs against distinct surface molecules suggesting that true cell fusion had occurred. We also evaluated the ability of human DC-TC hybrids to present tumor-associated epitopes in the context of both MHC class I and class II molecules. Allogeneic DCs expressing HLA-A*0201, HLA-DR.beta.1*0401, and HLA-DR.beta.1*0701 were fused with 888mel cells that do not express any of these MHC molecules, but do express multiple melanoma-associated Ags. DC-888mel hybrids efficiently presented HLA-A*0201-restricted epitopes from the melanoma Ags MART-1, gp100, tyrosinase, and tyrosinase-related protein 2 as evaluated by specific cytokine secretion from six distinct CTL lines. In contrast, DCs could not cross-present MHC class I-restricted epitopes after exogenously loading with gp100 protein. DC-888mel hybrids also presented HLA-DR.beta.1*0401- and HLA-DR.beta.1*0701-restricted peptides from gp100 to CD4(+) T cell populations. Therefore, fusions of DCs and tumor cells express both MHC class I- and class II-restricted tumor-associated epitopes and may be useful for the induction of tumor-reactive CD8(+) and CD4(+) T cells in vitro and in human vaccination trials.

AU Shimizu Keiji; Kuriyama Hideyuki; Kjaergaard Jorgen; Lee Walter; Tanaka Hiroshi; Shu Suyu
SO Journal of immunotherapy (Hagerstown, Md. : 1997), (2004 Jul-Aug) 27 (4) 265-72.
Journal code: 9706083. ISSN: 1524-9557.

TI Comparative analysis of antigen loading strategies of dendritic cells for tumor immunotherapy.

AB Dendritic cells (DCs) loaded with antigens can effectively stimulate host immune responses to syngeneic tumors, but there is considerable controversy as to which forms of antigen-loading are most immunogenic. Here, the authors compared immunotherapeutic reactivities of DCs loaded with a variety of antigen preparations. Because DC maturation stages affect their capacities of antigen processing and presentation, two DC populations were used for the current analysis: in vivo Flt-3 ligand-induced mature DCs and in vitro bone marrow-derived DCs, which were less mature. To facilitate a direct comparison, the LacZ gene-transduced B16 melanoma model system was used, where beta-galactosidase served as the surrogate tumor-rejection antigen. DC loading strategies included pulsing with the beta-galactosidase protein, H-2K restricted peptide, tumor cell lysate, and irradiated tumor cells and fusion of DCs with tumor cells. Our results demonstrated that electrofusion of DCs and tumor cells generated a therapeutic vaccine far superior to other methods of DC loading. For the treatment of 3-day established pulmonary tumor nodules, a single intranodal vaccination plus IL-12 resulted in a significant reduction of metastatic nodules, while other DC preparations were only marginally effective. Immunotherapy mediated by the fusion cells was tumor antigen-specific. Consistent with their therapeutic activity, fusion hybrids were the most potent stimulators to induce specific IFN-gamma secretion from immune T cells. Furthermore, fusion cells also stimulated a small amount of IL-10 production from immune T cells. However, this IL-10 secretion was also induced by other DC preparations and did not correlate with in vivo therapeutic reactivity.

IN Kanz, Lothar; Walden, Peter; Stuhler, Gernot

SO PCT Int. Appl., 42 pp.
CODEN: PIXXD2

TI Hybrid cell vaccines derived by fusion of an allogeneic dendritic cells and a non-dendritic cells and uses in tumor and infection therapy

AB The present invention relates to methods and compns. for treating and preventing cancer and infectious disease using hybrid cells formed by fusion of allogeneic dendritic cells and autologous non-dendritic cells which shares at least one class I MHC (major histocompatibility complex) allele. Such hybrid cells combine the vigorous alloreactivity of mature dendritic cells with the specific antigenicity of autologous tumor cells, thereby eliciting a highly specific and vigorous cytotoxic T lymphocytes (CTL) response. The invention also provides the methods for making hybrid cell vaccines and evaluating its cytotoxicity. For rapid and large-scale generation of hybrids, electrofusion is established as a two-step procedure: in the first step, tumor cells and dendritic cells (DCs) were dielectrophoretically aligned to from cell-cell conjugates; in the second step, a fusion pulse was applied, yielding 10-15% hybrid cell formation. The invention demonstrates that vaccine with tumor cell-dendritic cell hybrid results in regression of human metastatic renal cell carcinoma.

AU Tanaka H.; Shimizu K.; Hayashi T.; Shu S.

SO Cellular Immunology, (2002) 220/1 (1-12).
Refs: 53
ISSN: 0008-8749 CODEN: CLIMB8

TI Therapeutic immune response induced by electrofusion of dendritic and tumor cells.

AB To elicit a therapeutic antitumor immune response, dendritic cells (DCs) have been employed as a cellular adjuvant. Among various DC-based approaches, fusion of DCs and tumor cells potentially confers not only DC functionality, but also a continuous source of unaltered tumor antigens. We have recently demonstrated successful generation of fusion hybrids by a large-scale electrofusion technique. The immunogenicity and therapeutic

potential of fusion hybrids were further analyzed in a model system of a murine melanoma cell line expressing .beta.-galactosidase (.beta.-gal) as a surrogate tumor antigen. A single vaccination with fusion hybrids plus IL-12 induced a therapeutic immune response against 3-day established pulmonary metastases. This immunotherapy was .beta.-gal specific and involved both CD4 and CD8 T cells. In vitro, fusion hybrids stimulated specific IFN-.gamma. secretion from both CD4 and CD8 immune T cells. They also nonspecifically induced IL-10 secretion from CD4 but not CD8 T cells. Compared to other DC loadings, our results demonstrate the superior immunogenicity of fusion. The current technique of electrofusion is adequately developed for clinical use in cancer immunotherapy.

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